



Review

Where systems biology meets postharvest

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ABSTRACT

According to the dictionary, a system is something like “a group or combination of interrelated, interdependent, or interacting elements forming a collective entity”. In postharvest, fresh harvested food crops can be considered isolated small scale systems. Postharvest research aims to understand the quality of these ‘systems’ as influenced by postharvest conditions. The phenotypic quality of horticultural produce is based on genetic traits that are expressed through a cascade of reactions subject to complex regulatory mechanisms and diverse environmental conditions. Ultimately, to fully understand postharvest phenomena, a systemic approach that links genetic and environmental responses and identifies the underlying biological networks is required. Thanks to the development of high throughput omics techniques such system-wide approaches have become a viable option to support traditional postharvest research. This review provides an overview of systems biology and how it can lead postharvest research into a new era.

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1. Biological systems

The early years of biology have been influenced by two important concepts. The concept of reductionism goes back to Descartes (1596–1650) and holds that a complex system is nothing but the sum of its parts, and, therefore, can be understood by the behaviour of its constituents. The concept of mechanism additionally states that all natural phenomena can be explained and are determined by fundamental laws of nature (Trewavas, 2006). Early last century a more holistic approach was introduced stating that behaviour of a given system cannot be explained by its constituents alone. Instead, the system, as a whole, may override and largely determine how the parts behave (Trewavas, 2006). This goes back to Aristotle (384–322 BC) who stated that the whole is more than the sum of its parts. In contrast to machines, biological systems are inherently prone to, and capable of coping with, large sources of biological variation, undermining the strict deterministic mechanistic approach. At the same time (1920–1940), an interdisciplinary approach was developed for the biological sciences by Von Bertalanffy and Weiss (Drack et al., 2007). Von Bertalanffy's General Systems Theory provided the base for the current field of systems biology. The essential revolution came from the realisation that biological systems are hierarchically organised with influences going both up and down through the hierarchy. Feedback control mechanisms typically contribute to maintenance of homeostasis while feed-forward mechanisms are typically involved in inducing robust changes in systems behaviour (Kitano, 2001). The existence of such complex control systems makes the whole more than the sum of the parts and warrants the existence of the integrative approach advocated by modern systems biology.

Systems biology is the field of research that aims to understand complex biological systems at the systems level (Kitano, 2001). A system can be seen as a set of interacting or interdependent entities, forming an integrated whole serving a common objective. Biological systems are real-life complex systems that serve common goals of survival and ultimately reproduction (Koshland, 2002). Like any other system, biological systems are characterised by (i) a given structure, with (ii) a given behaviour and (iii) interconnectivity (Dubrovsky, 2004).

The structure of a biological system is defined by its physical parts (e.g., tissues, cells, organelles) and their composition (e.g., DNA, proteins, metabolites, lipids). Their behaviour involves inputs (e.g., external stimuli such as light, temperature, atmospheric composition, pH and nutrient levels and internal stimuli such as proteins, metabolites, hormones and various other compounds

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that might act as signal molecules), processing (e.g., via catabolic and anabolic pathways, or processes such as gene expression, differentiation and cell division) and outputs of material (e.g., proteins, metabolites), information (e.g., transcripts) or energy (e.g., heat, ATP, movement). Finally a biological system is characterised by a high degree of interconnectivity between its various parts, showing both functional relationships (e.g., through metabolic, signalling and gene regulation pathways) and structural relationships between each other (e.g., through compartmentalisation, receptor molecules, membrane transporters, plasmodesmata, vascular tissue, and the cytoskeleton).

Robustness is an essential property of biological systems (Kitano, 2001). Several concepts contributing to robustness known from engineering systems can also be recognised in biological systems. Control schemes, such as feed-forward and feedback controls, are primarily responsible for actively controlling the desired status of a system (Freeman, 2000). Redundancy is a second concept (Mantovani, 1999), where, for example, isogenes and gene replication allow systems to become more robust in response to gene or allelic damage. Complementary metabolic pathways also contribute to cell survival against environmental or genetic change (Lee et al., 2005). Modular design is inherent to multi-cellular biological systems and eukaryotic cells where compartmentalization predominates. The modular design reflects the hierarchical structure of biological systems and contributes to robustness by preventing a local failure from spreading to the wider system. Finally, structural stability contributes to robustness where, the structure of the control system largely determines the system behaviour by being highly tolerant to fluctuations in the different control parameters (Freeman, 2000).

2. An integrative approach

Systems biology relies on a multidisciplinary approach to integrate data from various disciplines of biology (Friboulet and Thomas, 2005) bringing together molecular disciplines (e.g., genetics, biochemistry, molecular biology) with those involving more complex systems (e.g., cell biology, microbiology, plant or human physiology). The aim of systems biology is to link the quantitative data in a mathematically defined sense across the different scales of biological organization (from DNA, RNA, protein to cell, tissue, organs). Mathematical modelling is used to drive integration with an aim of reaching a unified understanding of biological systems (You, 2004). Next to applications in human physiology, systems biology has already been embraced by various disciplines ranging from ecology (Benfey, 2004), crop physiology (Yin and Struik, 2008), to organismal biology (Schwenk et al., 2009), microbiology (Park et al., 2008) and molecular cell biology (Anonymous, 2006; Westerhoff and Palsson, 2004). The goal of systems biology therefore is to define the structure, dynamics and control of biological systems. The expected impact from systems biology focuses on an improved integrative understanding of biological systems (Kitano, 2001), to subsequently allow for better control and prediction of the behaviour of existing biological systems (e.g., improved human disease treatments; (Westerhoff and Palsson, 2004)) and then to mediate the design of new biological systems with desired properties (e.g., Dixon, 2005; Drubin et al., 2007; Sweetlove et al., 2003).

2.1. Systems thinking

The current renewed interest in systems thinking has been driven by the tremendous increase in data collected through the various high throughput 'omics'-techniques (Kandpal et al., 2009; Suwabe and Yano, 2008) associated with the failure of classical reductionistic approaches to interpret systems behaviour from its

constituents (Kitano, 2001). While genomics and associated bioinformatics data handling in genome projects such as the human programme and those of crops such as tomato, grape, rice, wheat, maize, potato and apple have been responsible for generating new insights into important constituents and structures of biological systems, this is not sufficient to create understanding of the functioning of the system impacted by changing conditions. The various forms of communication that operate within the hierarchy of a system are essential to understanding the overall systems behaviour (Trewavas, 2006). These relationships are not likely to be obtained from straightforward profiling experiments in any of the 'omics' areas but, instead, have to be deduced from carefully designed dynamic perturbation experiments in which the response of the system to changing environmental conditions is monitored.

2.2. Top-down versus bottom-up

Within systems biology two complementary approaches have been used: top-down and bottom-up (Bruggeman et al., 2007; Bruggeman and Westerhoff, 2007; Kitano, 2001). Top-down approaches start from system-wide data collected through the various high throughput 'omics'-techniques to define the time dependent composition of the physical parts of the system structure and apply inductive data analysis techniques (e.g., bioinformatics, statistical models, neural networks, Bayesian models) to identify global network structures based on phenomenological correlation data. This can lead to the identification of new, but still virtual, molecular mechanisms and the formulation of hypotheses concerning their regulation and other interactions. The major challenge for the data analysis is to integrate 'omics' data coming from different organisational levels to develop hypotheses on cellular regulation in terms of signal transduction, feedback mechanisms, etc., instead of only inferring networks at the level of genes (de la Fuente et al., 2002). The top-down approach is thus a data-driven and mainly a phenomenological based approach with emphasis on high throughput data collection resulting in models which are merely qualitative (Fig. 1).

Eventually, top-down approaches can feed into bottom-up approaches once the responsible mechanisms have been properly identified. Bottom-up approaches rely on deductive modelling techniques where the models are deduced from established views and fundamental relationships rooted in theories of, for example, chemistry and physics ('first principles'). Small scale experiments are conducted to calibrate and validate models on defined parts of the larger system and subsequently to challenge hypotheses derived from these models (Slepchenko et al., 2003). Ultimately, pathway models of a detailed mechanistic level should be combined with signalling and regulatory networks to come to a mechanism based model at the systems level. The bottom-up approach is thus a hypothesis-driven and mainly a mechanism based approach with emphasis on computational modelling techniques resulting in quantitative models (Fig. 1).

2.3. Systems biology work flow

Systems biology will never be a purely top-down or bottom-up approach (Goodacre et al., 2004; Kitano, 2002) but the two will be combined as indicated in Fig. 1. As in all sciences, fundamental and applied research will be driven by a certain research question. This research question will concern a certain (sub)system that has to be defined to some level by setting its boundaries to discriminate between what is and what is not part of the (sub)system under study. If the research question is formulated with a conceptual model in mind, some hypothesis can be formulated and more or fewer experiments can be designed to challenge the hypoth-

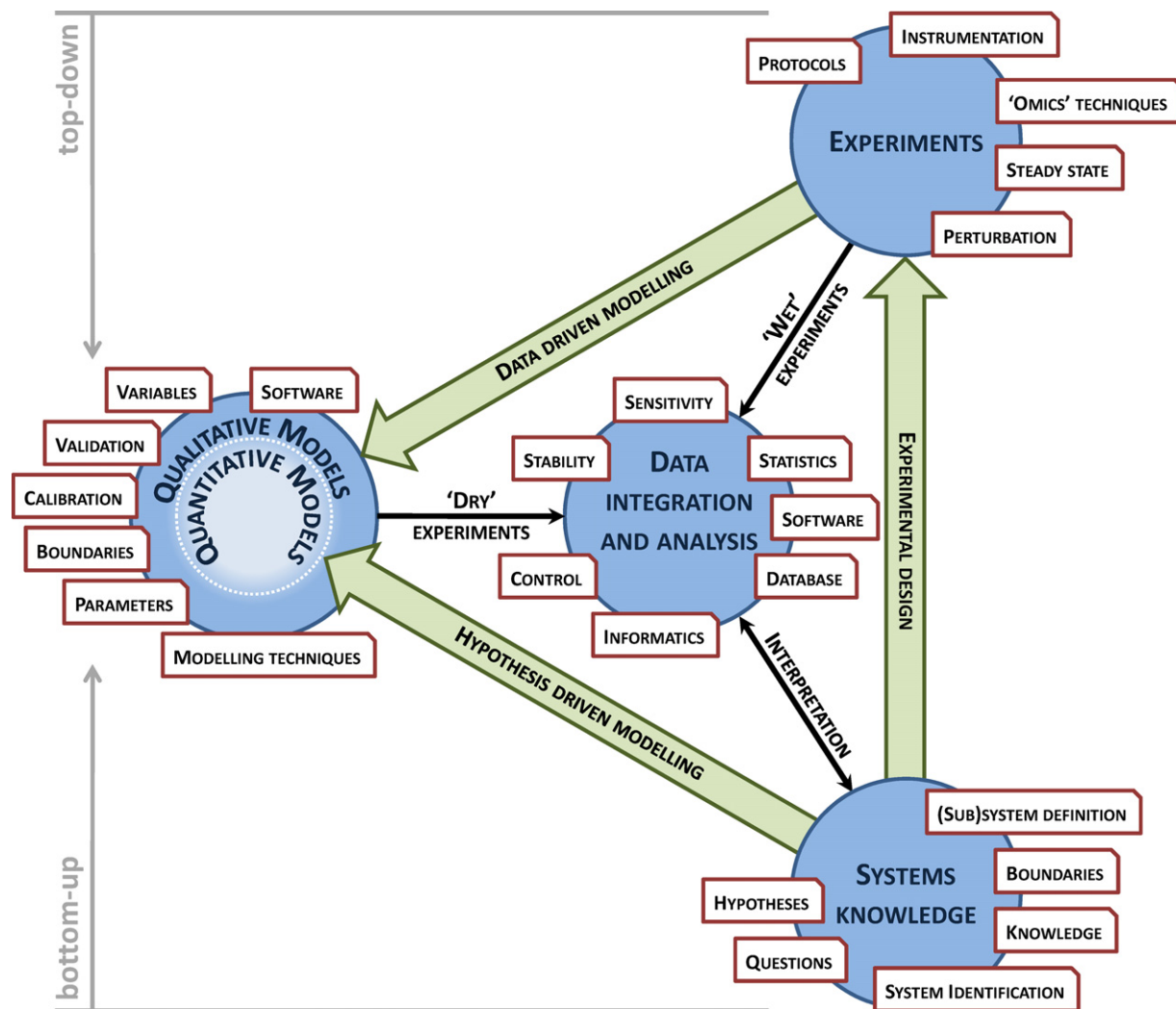


Fig. 1. Schematic outline of the systems biology work flow. The top-down approach relies on a holistic data-driven modelling approach while the bottom-up approach relies on a hypothesis-driven approach. This schematic outline combines insights derived from Goodacre et al. (2004), Ideker et al. (2001), Kim et al. (2008), Kitano (2001, 2002) and Wolkenhauer (2007). For further explanation see text.

esis by deductive reasoning. If no conceptual model is available, no clear hypothesis can be formulated and holistic experiments need to be designed to collect system-wide information to generate new hypotheses through inductive reasoning. By iteratively going through the cycle of designing new experiments to generate and challenge new hypotheses, the mathematical and conceptual models can be shaped, improved, and applied to create insight in and understanding of the structure, behaviour, and control of the biological system under study.

Models can be developed at any scale and with any level of complexity using a range of modelling techniques some of which will be discussed separately below. However, what all models have in common is that the model structure needs to be defined in terms of the model boundary, dependent and independent variables and model parameters (model identification; Oreskes et al., 1994). Subsequently the unknown parameters need to be given numerical values (model calibration) based on literature or information rich experimental data. To test the applicability of a model a validation is required which is preferably independent. However, one should realise that even a perfect validation does not provide a guarantee that the abstracted model is actually “true” under all conditions (Oreskes et al., 1994). Therefore, the model itself should never become the aim, but should remain a tool to create added value by challenging existing concepts and theories. The insights

gained from large scale system models are not always obvious and can be counter intuitive revealing “hidden features” when applied to new unique conditions (Kolch, 2008).

Data integration and analysis is the central hub in the systems biology work flow (Fig. 1) if only for its important role in data standardisation to allow for efficient handling and exchange (Suwabe and Yano, 2008). Over the years, much effort has gone into bioinformatics, statistics and computational biology (De Moor et al., 2003) to store, catalogue, condense, and mine the huge experimental datasets generated. In addition, various techniques (e.g., sensitivity, stability, bifurcation and control analysis; Kitano, 2001) are combined in evaluating data from simulation experiments to study robustness of the biological system modelled (Wolkenhauer, 2007).

Experiments, in a systems biology context, are characterised by high throughput ‘omics’ techniques, some of which are summarised in the following sections. These techniques have been made possible through rapid technological advances in modern laboratory instrumentation. While steady state experiments are of interest to identify the structure of biological systems, emphasis has shifted towards perturbation experiments to unravel the dynamic control of systems (Ideker et al., 2001). These perturbations can include gene alterations, changes in environmental conditions or hormonal stimuli.

3. Technologies for postharvest systems approaches

The current explosion of data from high throughput and global analyses has opened up new possibilities in systems modelling. As more comprehensive data are collected on gene transcription, translation and consequent metabolism, it is obvious that developing novel ways of connecting pathways and data at the various levels of organisation are critical in developing our understanding of the physiological responses of a biological system. The complexities of the complete flow of information through transcription, translation and into metabolic response are only just being appreciated. The following sections provide an overview of our understanding of these levels of organisation in a postharvest context.

3.1. Genomics and transcriptomics

Genomics is the acquisition and analysis of DNA sequence data. While initially genomics was used to describe large scale gene sequence, with recent advances in molecular techniques, genomics has now come to describe a more global approach to understanding DNA structure. Early genomic approaches were based on ESTs (Expressed Sequence Tags) which are single reads from complementary DNA (cDNA) derived from messenger RNA (mRNA) expressed at the time of sampling. These single reads can be aligned with each other through bioinformatics into recognisable gene sequences giving valuable gene information. These genomic studies typically generated large numbers of ESTs, but now a wider and deeper analysis of gene families and expression has become possible, including increased facility to identify regulatory gene networks and the control of expression.

Transcriptomics arose from the combination of genomics data and miniaturisation, allowing the creation of microarrays and gene chips containing 1000s of known gene sequences. The presence of mRNA corresponding to each of these sequences could be measured from different tissues or different sampling events, generating knowledge of where and when a gene is expressed, and giving a snapshot of the transcriptome in one experiment. The limitations of this technology were the need for gene sequence to create the microarrays. Once created, only expression information of the genes selected could be measured. Hybridisation technology is now being overtaken by a naïve sequencing technology that overcomes the prior need for gene models. The development of pyrosequencing and imaging technologies, have allowed sequencing to be performed and measured at microscopic levels. Plates or arrays containing millions of reactions can be assayed at a single time. Platforms such as Roche FLX, Illumina Hi-Seq and SOLiD Technologies are now available, and this technology has been utilised for transcriptomics by frequency counts of transcripts within pools of cDNA. This has huge advantages, as every gene that is expressed can be interrogated, the splice variation in a population can be measured, and dynamic range is not affected by saturation of a fluorescent probe at the high end and background fluorescence at the low end. As sequencing technology is rapidly getting cheaper it is becoming the transcriptomic technology of choice among researchers.

3.1.1. State of the art

The success of genomic approaches depends on sequence databases and attendant bioinformatics systems. To date, EST databases for fruit such as grape (Moser et al., 2005), pineapple (Moyle et al., 2005), citrus (Forment et al., 2005; Maul et al., 2008), apple (Newcomb et al., 2006; Park et al., 2006) and kiwifruit (Crowhurst et al., 2008), have been made publicly available and various levels of analysis published. Most of these large databases have been developed from a range of plant tissues, including vegetative

and reproductive organs, and are not specifically targeted at fruit ripening. However, those such as apple (Newcomb et al., 2006) and kiwifruit (Crowhurst et al., 2008) have included ESTs from libraries of ripening fruit both on the tree and postharvest. With kiwifruit for example, this has enabled mapping of cell wall genes over various stages of fruit softening over 10 days postharvest ripening (Crowhurst et al., 2008). ESTs generated from different species or cultivars are also a significant source of material, such as single nucleotide polymorphisms (SNPs), for constructing genetic maps, resulting in markers for traits important for postharvest quality. Integration of ESTs, genetic maps, and whole genome sequence information with breeding programmes is proving to be of major benefit in developing new cultivars with improved postharvest quality and response characteristics (Chagne et al., 2007, 2008).

More recently, whole genome sequences for fruit species have started to become available, such as those published on apple (Velasco et al., 2010), grape (Jaillon et al., 2007), papaya (in this case a virus-resistant transgenic variety; Ming et al., 2008), and strawberry (Shulaev et al., 2011) while those of tomato and peach are likely to appear soon. These have the advantage of providing sequence data independent of expression at the time of sampling, and allowing a more complete analysis of gene families and pathways.

Microarrays heralded a new era allowing the measurement of gene expression of multiple genes simultaneously. These data, however, have limitations as they require validation through alternate expression technologies (e.g. quantitative PCR) and they depend on the accuracy of gene annotation in public databases. The availability of whole genome sequences has allowed for the development of microarrays that cover the complete transcriptome and even representation of the continuous DNA strand by tiling arrays providing the means for a deeper analysis of the transcriptome, including cytosine methylation and identification of polymorphisms (Gregory et al., 2008). The ability to sequence millions of transcripts at a time, is currently allowing researchers to move further away from mainstream species and interrogate less well studied organisms to answer specific questions (Alagna et al., 2009). We expect this to be the case with fruit and vegetable crops of significant postharvest interest.

High throughput qPCR has also made small scale transcriptomics of single gene families accessible, such as studying the dynamics and patterns of lipoxygenase (LOX) gene expression (Zhang et al., 2006) or ethylene signalling gene expression (Tacken et al., 2010; Yin et al., 2010, 2009, 2008) during ripening. This approach has proven particularly valuable in identifying and characterising low-expressed regulators e.g. transcription factors that might not have been detected using hybridisation technologies, such as the identification of the molecular control of red colour development by the MYB10 transcription factor in ripening mandarin fruit (Palapol et al., 2009).

3.1.2. Current use in postharvest

The ultimate goal of a genomics approach to postharvest biology is to understand the molecular control of postharvest responses include general processes such as ripening and senescence. Understanding the molecular regulation of these processes could lead to better technologies for controlling the physiological processes associated with ripening and, potentially, storage disorders.

It is only relatively recently that specific postharvest experiments have been carried out using genomic approaches. This is likely to increase with the increasing availability of sequence databases and more sophisticated expression technology such as tiling arrays. Arrays have been used to studies on ripening changes in peach, apple and nectarine (Janssen et al., 2008; Schaffer et al., 2007; Trainotti et al., 2006; Ziliotto et al., 2008), postharvest water loss in grapes (Rizzini et al., 2009) and on carbon dioxide response of

strawberries (Ponce-Valadez et al., 2009) and grapes (Becatti et al., 2010). In addition, cold responsive genes have been identified in peach (Tittarelli et al., 2009).

Transcriptome array analyses show that mutation influences expression of more than 30% of genes differentially expressed during tomato fruit development and ripening (Alba et al., 2005). The complexity of the ethylene signalling pathway from receptors through to transcription factor families is being well-characterised in tomatoes and the availability of the whole genome sequence for tomato will likely produce more family candidates. For example, more than 100 ERF-like genes have been identified in Arabidopsis and rice (Nakano et al., 2006). Co-ordinated expression studies of gene families within such pathways are now starting to appear, as for ripening kiwifruit, with 5 receptors, 2 CTR-like genes, 4 EIL-like genes and 14 ERF transcription factors analysed in single experiments (Yin et al., 2010, 2009, 2008).

The availability of arrays has allowed a more global approach to texture. For example, the use of a peach fruit array has shown substantial numbers of differentially expressed genes associated with different levels of ripening and softening of nectarine fruit treated with 1-MCP (Ziliotto et al., 2008). Softening in strawberry fruit has been well-dissected through the use of antisense technology (e.g., Quesada et al., 2009; Santiago-Domenech et al., 2008) and the availability of whole genome sequence for this species will enable a wider perspective on softening control.

Other major traits or responses associated with postharvest responses are more difficult to analyse through single gene approaches. Flavour development and low temperature response are multigenic, and thus lend themselves well to approaches which involve global expression analysis based on EST or whole genome sequences. For example, the involvement of LOX genes in aroma development in tomato fruit has been painstakingly analysed using antisense in tomatoes to determine which LOX gene had a measure of control over flavour (Chen et al., 2004a). Use of an EST database and *in vitro* expression allowed 6 LOX members to be much more quickly analysed in ripening kiwifruit, showing differential expression and association with hexanal and hexenal production (Zhang et al., 2006, 2009). An apple array together with an ACO antisense transgenic apple line has been used to show the regulatory role of ethylene in the biosynthesis pathways of aroma volatiles (Schaffer et al., 2007). In low temperature response, typically some hundreds of genes can be shown to respond to chilling conditions; in grapefruit low temperature treatments elicited gene response to both tolerance- and injury-inducing conditions (Maul et al., 2008).

3.1.3. Limitations and opportunities

While microarray studies focus on relative amounts of mRNAs in the cell, these are not necessarily proportional to the expression level of the proteins they code for. This is largely regulated by translation-initiation features of the mRNA sequence. Microarray analyses of postharvest responses, while providing interesting comparative information on gene types, still require more detailed expression and functionality studies before co-ordinated gene function can be confirmed. A major limitation of these microarrays are that the genes that are represented on the arrays are often not chosen from EST libraries derived from postharvest treated fruit. This again can be addressed by accessing whole genome sequences or a second generation sequencing approach not relying on pre-existing knowledge of genes to be tested.

The use of expression data in isolation for systems modelling will be problematic. PCR- or deep sequencing-based expression data of members of specific gene families, while providing comparative data for individual family members, highlight the gaps in understanding information flow. For instance, for kiwifruit ethylene signal transduction (Yin et al., 2010, 2009, 2008), 5 receptor, 2 CTR, 4 EIL and 14 ERF genes were isolated and their expression

under normal ripening in air, stimulation by external ethylene, treatment with 1-MCP and treatment with low temperature evaluated. Within each of those sets of genes, different expression patterns were found under the various conditions, overall providing several hundred different pieces of specific expression data. Although the information flow from receptor to CTR to EIL and ERF has a linear component, the expression data do not specifically provide a sequential flow of information, but rather a snapshot of gene levels at each sample time. By knowing what the relative expression levels are at, say, 48 h after harvest under ethylene treatment at 20 °C for all 24 genes, the data provide a comparative picture. However, the dynamics are missing. This is even more obvious when considering that the link between RNA level and associated protein is almost completely unknown. What little data there are available suggest that no assumptions can be made. For instance, transcripts of some ethylene receptors in ripening tomato fruit have been shown to increase concomitant with a decrease in receptor protein level (Kevany et al., 2007) and similar differences between RNA and protein levels for receptors in response to ethylene have been shown in apple fruit (Tatsuki et al., 2009). Understanding and quantification of gene expression is further confounded by epigenetic mechanisms, where developmental and environmental impacts on DNA can lead to greater levels of variability in expression (Seymour et al., 2008).

One of the greatest limitations in the postharvest arena is that much of the work is done on woody fruit crops such as apple, grape, citrus etc. Transgenic approaches to elucidate gene function can take a considerable time, persuading researchers to use model heterologous systems such as Arabidopsis or tomato, and transient expression systems such as developed by Hellens et al. (2005) in tobacco. The other major limitation is the nature of multigene effects in postharvest responses such as those to low temperature or low oxygen. The sensing mechanisms for these conditions are still largely elusive and elucidation of the critical gene control mechanisms is difficult. The most advanced systems approach to postharvest issues may well come from peach, where transcriptomic (e.g., Lazzari et al., 2008; Tittarelli et al., 2009; Vizoso et al., 2009), and proteomic (e.g., Lara et al., 2009; Nilo et al., 2010; Zhang et al., 2010) data are being accumulated. The biggest limitation may be in our current ability to combine and model the information. Ultimately next generation sequencing technology will not only lead to genomic sequencing of non-model species, it will also allow the sequencing of multiple genomes, allowing comparisons of diverse individuals in germplasm collections. This is already been achieved in the model plant Arabidopsis, where there is current sequence availability for multiple ecotypes, with the current plan to sequence 1000 individuals to identify genetic diversity. This is yet to have an impact on plant gene regulation studies, let alone the postharvest world.

3.2. Proteomics

Proteomics is the study of the whole set of proteins encoded by a genome. The goal of proteomics is to understand at a given time and under specific conditions, how proteins change in terms of expression, structure and function; proteins being the key to metabolic processes. Proteomics as a platform, provides information on regulation of metabolism, and besides delivering biological markers, also delivers targets of intervention (Kusmann et al., 2006).

3.2.1. State of the art

A typical proteomics work flow consists of protein extraction, protein or peptide separation and quantification, protein identification and data analysis and integration. Protein extraction and precipitation from plant materials can be challenging due to the existence of interfering compounds such as phenolics, pigments

and oxidative enzymes. Specific methods such as phenol extraction precipitation in methanol ammonium acetate have been successfully used with fruit material (Carpentier et al., 2005; Pedreschi et al., 2007).

To separate proteins or peptides, two main approaches exist: gel-based and gel-free differing in the way proteins are isolated, separated and detected. Both approaches are thus complementary and focus on a subset of proteins. In a gel-based approach, proteins are first separated by means of two dimensional electrophoresis (2-DE) based on isoelectric point and molecular weight properties of proteins. Protein spots can be visualised by silver or Coomassie Brilliant Blue staining. A 2-DE involves labelling the proteins before separation is carried out. This sophisticated technology is known as differential gel electrophoresis (DIGE) and up to three samples can be resolved on a single gel, thus reducing technical variability while increasing confidence in terms of detection and quantification (Unlu et al., 1997). After separation, proteins are independently digested for further mass spectrometry identification.

In a gel-free approach, protein digests are analysed by means of LC-MS/MS and quantification can be carried out by labelling the peptides with stable isotopes (Gygi et al., 1999; Roe and Griffin, 2006; Ross et al., 2004). Both with gel-based or gel-free approaches, proteins need to be digested before being introduced into a mass spectrometer. In most cases trypsin is used to cleave proteins on the carboxy-terminal side of arginine and lysine residues (Steen and Mann, 2004).

A mass spectrometer uses an ion source to produce ions from the sample. For the analysis of proteins, two soft ionization techniques, matrix assisted laser desorption ionization (MALDI) and electrospray ionization (ESI) are used (Fenn et al., 1989; Karas and Hillenkamp, 1988). One or more analysers are needed to separate the ions based on their m/z ratios, a detector to register the number of ions coming from the last analyser and a computer to process the data and produce the mass spectra (Lane, 2005). Either peptide mass fingerprinting (PMF) typically performed by MALDI-TOF-MS or tandem mass spectrometry (MS/MS) coupled to LC are used for protein identification (Mathesius et al., 2002). For data analysis, either univariate or multivariate statistical approaches can be utilized but the focus and information extracted from such analysis may turn out to be different (Karp et al., 2005; Pedreschi et al., 2008, 2007).

3.2.2. Current use in postharvest

While there is a literature on conventional 2-D gel protein separation and putative identification in postharvest systems going back over several years, a true proteomic approach to fruit ripening has only appeared more recently in fruit such as tomatoes (Faurobert et al., 2007) and grapes (Giribaldi et al., 2007; Lucker et al., 2009; Zhang et al., 2008). Protein markers for detecting optimum harvest maturity (Abdi et al., 2002) and analysis of genotypic variation (Rocco et al., 2006) have resulted from such studies. Mechanisms involved in storage disorders such as core breakdown in 'Conference' pears, creasing in citrus, and blossom-end rot in tomato (Casado-Vela et al., 2005; Lliso et al., 2007; Pedreschi et al., 2008, 2007) have been studied using a gel-based approach. Proteomics tools have also been used to study physiological implications of disorders in tomato infected with mosaic virus (Casado-Vela et al., 2006), peach fruit infected with mould rot (Chan et al., 2007), and infected cherry fruit (Chan et al., 2008; Xu and Tian, 2008).

3.2.3. Limitations and opportunities

One of the limitations of the application of proteomics in postharvest science is the comparatively low number of horticultural crops with their genomes completed. Thus cross species identification is carried out by comparing the peptides of proteins

of interest with orthologous proteins from other well-characterised species. In addition, for non-completed genomes, only a gel-based approach is possible and this approach is certainly biased to resolve certain types of proteins (e.g., highly abundant, acidic and hydrophobic proteins are not resolved). With the increase in cheaper, next generation sequencing (see above), this situation is expected to change in the near future. There are target areas in postharvest responses where a proteomics approach might provide levels of insight not yet reached through more conventional approaches. These are particularly where responses or processes are multigenic.

Temperature control has always been a challenge in postharvest given that low temperature slows down ripening but if improperly applied in a specific commodity can result in a range of storage disorders. Temperature affects both membrane bound proteins and cytosolic enzymes (Galindo et al., 2007). Membrane proteins are one part of the proteome which are under studied and under estimated due to difficulties in extraction and characterisation (e.g., very hydrophobic, low abundant proteins). However, understanding the induction and behaviour of these proteins will be of key importance in designing storage technologies which minimise occurrence of temperature related disorders. Another area where there is an integrated gene and metabolic response is in texture changes in crops associated with changes in the cell wall (Vicente et al., 2007). Many cell wall polysaccharides and glycoproteins are synthesized in the Golgi apparatus and then trafficked to the cell surface. If the mechanisms involved in the synthesis of cell wall polysaccharides could be fully unravelled, then the selection or manipulation of desired textural properties would be feasible (Dunkley et al., 2006).

A number of other classes of proteins are becoming recognised as important in postharvest quality of produce, and proteomic approaches are likely to elucidate their roles more fully. For instance, a range of consumer attributes such as nutritional and health-related compounds are increasingly important in minimally processed horticultural crops, and we have limited information regarding the changes induced at a biochemical level. This includes induction of allergenic proteins which can easily be assessed by 2-DE and IgE immunoblotting. In addition, proteomic analysis is showing that classes such as pathogen-related (PR) proteins are frequently being identified in commodities under postharvest conditions, raising questions on their role in terms of fruit and vegetable quality and environmental response (Pedreschi et al., 2007; Sancho et al., 2006). The introduction of unintended effects resulting from biotechnology or processing in a crop can also be assessed using high throughput proteomic technology. In general, proteomics driven research is expected to offer great opportunities in the postharvest areas of process optimization and monitoring, quality and traceability, safety and nutritional assessment potentially contributing to innovations in the wider food industry (Pedreschi et al., 2010). However, currently the linkage to genomic and metabolomic information is poor to non-existent.

3.3. Metabolomics

Metabolic analysis has been an integral part of plant science and postharvest science for over a century. Metabolic profiling comprises a group of diverse techniques for evaluating one or more metabolites. "Metabolite profiling" typically refers to targeted analyses of specific metabolites or group of metabolites while "metabolomics" designates non-targeted and, optimally, non-biased, systemic analysis of the "metabolome" (Goodacre et al., 2004). Current comprehensive or global metabolic profiling protocols only evaluate a fraction of an estimated 15,000 metabolites of considerable variability in concentration, size, solubility, and stability within a single species (Allwood et al., 2008; Dixon,

2001). Instead, metabolic profiling or fingerprinting protocols are optimally non-targeted evaluations of metabolites from multiple pathways (Fernie, 2007; Goodacre et al., 2004); in effect, a representative sample of the metabolome. Plant scientists have adapted global profiling extraction and analysis techniques for evaluation of complex plant tissue matrices containing not only primary metabolites, but diverse classes of secondary metabolites of considerable importance to plant function as well as horticultural and postharvest value. The physical, temporal, and spatial resolution challenges presented by plant tissue require unique strategies for metabolomic estimation.

3.3.1. State of the art

Evolving instrumental and data analysis technology has presented new opportunities for increasingly more inclusive metabolomic estimation (reviewed by Allwood et al., 2008; Dunn et al., 2005; Fernie et al., 2004; Hagel and Facchini, 2008). The most prominent modern platforms include gas chromatography (GC), liquid chromatography (LC), or capillary electrophoresis (CE) coupled with mass spectrometry (MS), standalone MS, nuclear magnetic resonance (NMR) spectroscopy, and Fourier-transformation-infra-red spectroscopy. Recently, laser assisted micro-dissection (LAM, Moco et al., 2009) or various mass spectrometric techniques using laser desorption ionization (LDI, Sluszný et al., 2005) or colloidal graphite or infra-red matrix-assisted laser desorption ionization (GALDI, IR MALDI, Burrell et al., 2007; Cha et al., 2008; Li et al., 2008), and time of flight secondary ion mass spectrometry (TOF-SIMS, Metzner et al., 2008; Perkins et al., 2008) have been employed. Analytical platforms differ in metabolite selectivity, resolution, analysis speed, and potential throughput. Likewise, each extraction method imparts a certain degree of selectivity which may bias metabolomic estimation.

The improved analysis speed and coverage afforded using non-chromatographic techniques is typically at the cost of sensitivity and resolution (Goodacre et al., 2004; Hagel and Facchini, 2008). Additionally, GC–MS and LC–MS provide an extra degree of resolution over chromatographic systems. A number of software applications are available to uncover individual metabolites from these complex data matrices (Broeckling et al., 2006; Halket et al., 2005; Lommen, 2009; Smith et al., 2006; Tautenhahn et al., 2008; Xia et al., 2009). Metabolites are typically identified using spectral comparison with authentic standards or by using spectral libraries and metabolite databases (reviewed by Halket et al., 2005; Tohge and Fernie, 2009). Many features used for identification can be platform, technique, or even protocol specific and only libraries for a few platforms are useful for instrument-wide or universal comparison. Large metabolite libraries are available for the GC–MS, which typically employs a highly predictable electron ionization source, making it a particular popular platform for metabolite analysis and identification (Lisec et al., 2006). Also, GC retention standardization can be employed alongside mass spectral identification, for improved comparability (Kopka et al., 2005; Schauer et al., 2005a).

One of the most prominent plant tissue metabolomic analysis protocols employs GC–MS to analyse trimethylsilyl(oxime) derivatised aqueous extracts (Fiehn et al., 2000; Lisec et al., 2006; Roessner et al., 2000). This protocol has been optimized for a range of tissues including potato tubers (Dobson et al., 2008; Roessner et al., 2000; Roessner-Tunali et al., 2004), tomato fruit (Carrari et al., 2006; Schauer et al., 2005a), pear cortex (Pedreschi et al., 2009), and apple peel (Rudell et al., 2008; Rudell and Mattheis, 2009). While evaluating a broad range of primary metabolites, this protocol detects few secondary metabolites, many of which are essential to agricultural commodity quality as well as function. Similarly, protocols employed to measure secondary metabolites are targeted or focused, neglecting many primary and other

polar, non-volatile metabolites or physically diverse secondary metabolites (Hagel and Facchini, 2008). To compensate, multiple extraction and instrumental techniques can be employed to expand metabolomic estimation (Moco et al., 2007, 2008; Rudell et al., 2009).

3.3.2. Current use in postharvest

Storage, treatment, and distribution of perishable commodities all present a profusion of external stimuli having both positive and negative consequences on storability and quality. These can be examined using metabolomic evaluation techniques, yet to date, research employing untargeted metabolic profiling to study postharvest issues is relatively uncommon. Metabolomic protocols have been used to link changes in apple fruit quality-related metabolism and peel necrosis with varying pre-storage light environment (Rudell et al., 2008; Rudell and Mattheis, 2009). Metabolomic differences between pear cortex, with or without browning injury, have been evaluated (Pedreschi et al., 2009) as have links between apple peel necrosis (superficial scald), cold storage duration, oxidative stress, and ethylene insensitivity (Rudell et al., 2009).

3.3.3. Limitations and opportunities

Potential products of postharvest research using metabolomics are biomarkers indicative of important quality and supply chain factors such as ontogeny, surety of supply-chain quality, product safety, or pathogen infection. Already untargeted studies of volatile metabolites within storage head space have yielded prospective biomarkers indicating pathogen infection in a number of stored commodities (Lui et al., 2005; Vikram et al., 2005, 2006, 2004a,b).

Combining metabolomics with genetic approaches is a particularly promising application for determining heredity of biochemical traits and linking traits to specific genes (Fernie and Schauer, 2009; Goodacre et al., 2007; Keurentjes, 2009). Untargeted metabolic fingerprinting techniques can be used to distinguish individuals with unique quality characteristics within breeding and wild populations (Dobson et al., 2008; Schauer et al., 2005b; Stewart et al., 2007). While targeted metabolite analysis of mapping populations is useful for limited discovery of quality-related metabolic quantitative trait loci (QTL), untargeted biochemical phenotyping of genetically mapped populations can reveal multiple metabolic QTLs that comprise sensory traits (Dunemann et al., 2009; Zanor et al., 2009; Zini et al., 2005). This approach has been especially productive where genetic techniques, such as introgression, have been incorporated, effectively establishing metabolic trait heritability while unravelling metabolic control by genomic comparison instead of metabolic co-fluctuation with gene expression (Baxter et al., 2005; Keurentjes et al., 2006; Schauer et al., 2008, 2006).

Metabolomics is a product of advances in instrumentation and methodology which are not unfamiliar to postharvest science. New opportunities for improving perishable commodities' quality and nutritional value using breeding, for understanding storage related metabolism and its genetic control, for developing biomarkers to monitor storage physiology, and for improved food safety and quality assurance exist as a result of these new techniques. Most importantly, measurement of the metabolome has proven to have a crucial and complimentary role alongside the genome, proteome, and transcriptome in systems biology. All of these current and developing technologies will mean a stage will be reached rapidly where the ability to accumulate data is greater than the capacity to analyse them. This creates challenges for computational biology in general and the application and development of proper modelling tools in particular.

4. Modelling

A model is a simplified representation of real world objects or systems based on formalised assumptions of its structure, behaviour and interconnectivity; in other words, a model is a condensed tangible format of existing information (Ross, 1999). The level of model complexity required strongly depends on the intended purpose of the model. As models are by definition simplifications, they will never be “true” as the only true model is the system itself (Oreskes et al., 1994). Therefore, the aim of modelling is not to develop true models but to develop valid models; models that are consistent with the current knowledge level and that contain no known or detectable flaws of logic. Probably the main purpose of the modelling process is to force the researcher to line up his or her concepts to bring order to the chaos (Hertog et al., 2007).

The modelling process (Fig. 2) is an iterative process. The reason for these iterations is the learning aspect of stepwise increasing knowledge by experimentation, modifying the existing concepts, leading to subsequent model adaptations (Kreutz and Timmer, 2009). Essentially the same iterative procedure was observed in the systems biology work flow (Fig. 1). When there is uncertainty about the most likely model structure, simulated model behaviour can be compared to actual experimental data to discriminate between the different options (Cedersund and Roll, 2009). Once the most appropriate model is selected there might still be some unknown model parameters that need to be estimated from the experimental data. The identifiability analysis will help in designing targeted experiments that generate data containing enough information to estimate all unknown model parameters if possible. With increasing model complexity and many correlated parameters, a unique solution might no longer be available resulting in a non-identifiable model. As a result, individual parameter estimates will be unreliable although the overall model might still fit the experimental data.

4.1. Model approaches

The most rudimentary model is the conceptual model lurking in the back of the mind of an individual researcher, which can be schematically sketched on the back of a beer coaster to convey the concepts to eagerly listening students. As the complexity of biological systems surpasses the computational power of the human brain to process and organise the increasing amount of data, the largely qualitative conceptual model can be turned into more powerful quantitative mathematical models.

A wide range of such mathematical models has found an application in systems biology. The different approaches can roughly be separated into two groups of modelling techniques, inductive (Hills, 2001) and deductive (Verdenius, 2001). With inductive modelling techniques the model is induced by the data and no explicit expert knowledge is required. These techniques are completely data driven and might result in relationships with a limited validity. Examples of these techniques are multivariate statistical techniques such as MANOVA (Hwang and Park, 2009), PLS-DA (Perez-Enciso and Tenenhaus, 2003) and data mining techniques such as neural (Almeida, 2002) or Bayesian networks (Wilkinson, 2007). Deductive modelling techniques are based on explicit expert knowledge and are constructed using fundamental laws and generally valid relationships. Examples of these techniques are models on heat and mass transfer (Nicola et al., 2001), reaction diffusion models (Ho et al., 2008, 2006), biochemical kinetic models (Demin and Goryanin, 2008) and models of population dynamics (Ferrer et al., 2008). In systems biology, both types of models are encountered.

A major step in model development is the choice of the organisational level at which the model should be developed (e.g., cellular,

tissue, organ, organism) and which parts and processes (e.g., genes, metabolic pathways, or processes like gene splicing and cell division) should be included and which should be left out (Mogilner et al., 2006). What is essential and what is redundant largely depends on the intended application of the model; what should it be able to predict? Going through this modelling process, one is forced to consider functional and causal relationships. Within systems biology the tendency is to include as much data as possible and leave it to inductive techniques to determine which of the variables considered might be important. In a later stage, research focuses on identification and confirmation of pathways and control mechanisms starting from the inferred networks, evolving towards mechanism based models providing insight in the underlying interactions, molecular or otherwise (Belostotsky and Rose, 2005). However, the model complexity (e.g., number of variables and parameters, structural complexity in terms of compartmentalisation) has to be in line with the amount and type of data measured; for instance, it will be difficult to build a model on the genetic regulation of fruit ripening on metabolic data only.

4.2. Network based models

In a first instance, one would like to interpret the experimental data coming from any of the mentioned omics approaches in the light of known metabolic pathways as documented in for instance the KEGG Pathway Database (Kanehisa and Goto, 2000). These pathways serve as conceptual models that act as a reference framework when interpreting new experimental data. Over the years tools have been developed, such as MapMan, to visualise large scale omics data by projecting it on known pathways, thus making the numerical data accessible in a more user friendly format (Osuna et al., 2007; Usadel et al., 2009). While this approach is helpful in visualising data for established pathways, it is not generating quantitative data on regulation and control of the pathway nor will it actively help in identifying new pathways.

A wide range of approaches exists to model genetic regulatory systems ranging from probabilistic via Boolean, rule based and logical techniques to quantitative differential equations, either or not spatially distributed (De Jong, 2002; Hecker et al., 2009). Each of these techniques have their own constraints in how they describe the network by being either static or dynamic, discrete or continuous, deterministic or stochastic, qualitative or quantitative and by their different level of detail. Using these techniques, models can be either composed from knowledge on regulatory interactions (structural information) or induced from expression data (functional information) with both techniques being complementary.

With evolving computational power and growing databases, approximate qualitative models are being replaced with more detailed quantitative models (Wolkenhauer et al., 2005). Additionally, large efforts in the area of bioinformatics go towards techniques for effectively mining and combining pathway information from the various omic databases to support construction of large scale computational models of cellular processes (Cary et al., 2005; Price and Shmulevich, 2007; Yuan et al., 2008). The generated network models graphically represent metabolic pathways showing nodes, representing genes, RNAs, proteins or metabolites and their interrelationships represented by the arrows or lines connecting the nodes. Examples of gene regulatory networks that have been modelled for higher plants include flower development, circadian clocks and auxin fluxes during plant development (Long et al., 2008). Such network models can be used, starting from the known pathways, to generate coherent hypotheses which can be subsequently verified using experimental data (Rho et al., 2008). In addition, database information can be used to reconstruct networks for systems that have not been studied yet (Libourel and Shachar-Hill, 2008).

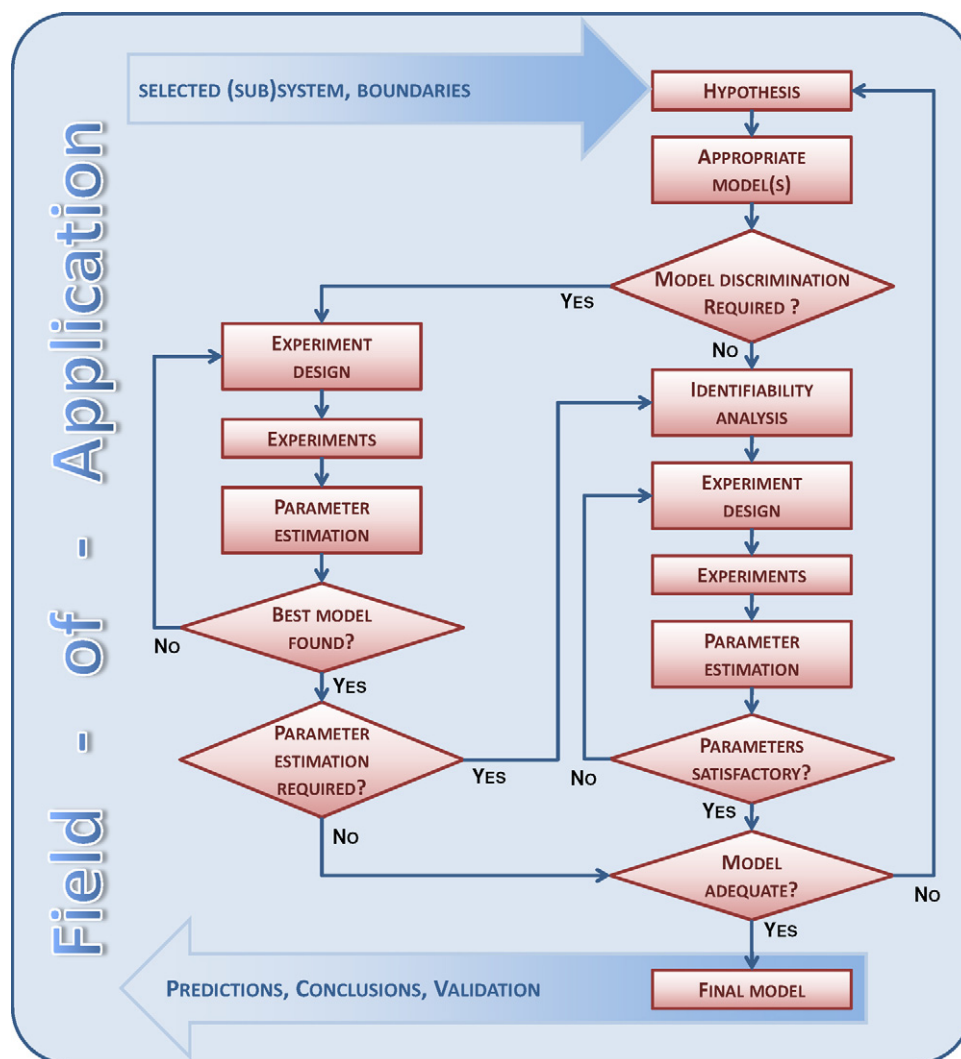


Fig. 2. Schematic outline of the model building process (adapted from Kreutz and Timmer, 2009). For explanation see text.

To discover new pathways various types of correlation analysis can be applied, for instance, to infer metabolic networks directly from experimental omics data (Goodacre et al., 2004). By using correlation network plots, the clusters of, for instance, correlated metabolites can be identified, and changes in correlation strengths can be interpreted in the light of functional and regulatory relations between the metabolites (Ursem et al., 2008). Such network models can be derived using unsupervised techniques, such as hierarchical clustering and principal component analysis, that search for similar correlation patterns in the omics data. Alternatively supervised techniques can be used, such as discriminant analysis, partial least squares regression and artificial neural networks, that additionally search for associations of the omics data with certain response traits. Although the correlation structure is a direct fingerprint of the biological system it is not that straightforward to reconstruct the actual reaction mechanism based on the correlation structure only (Steuer et al., 2003). Established metabolic pathways or reconstructed *in silico* network models can subsequently be used as a base for developing kinetic models and performing metabolic flux analyses using the stoichiometry of the network models.

4.3. Kinetic based models

Kinetic models provide a very detailed framework to describe cellular processes in terms of chemical reactions. In this concept the

status of the system is defined by the concentrations of the constituting compounds. These can either interact with other compounds being converted to new ones, or interact with other compounds regulating or influencing their turnover (e.g., in the case of enzymes, cofactors or competing compounds). They can be transported to other cellular compartments where they might be stored, act as a signalling molecule to trigger other processes, or become activated to perform some other task.

In the area of metabolic engineering, emphasis is on stoichiometric models of metabolic pathways which are founded in the reaction biochemistry assuming dynamic steady states (Stephanopoulos et al., 1998). A number of the tools developed for metabolic engineering, such as metabolic control analysis and metabolic flux analysis (Stephanopoulos et al., 1998) are therefore dedicated to analyse stationary states although some of these can also be applied to oscillatory systems as long as average fluxes are considered (Schuster et al., 2002). However, biological oscillations cannot be fully understood using only steady state assumptions as they operate through dynamic interactions between multiple biological processes. Two other techniques to study metabolic network models are extreme pathways analysis and elementary mode analysis (Libourel and Shachar-Hill, 2008). Both techniques aim to identify small sub-networks in order to study the regulatory mechanisms of a metabolic network in more detail (Price et al., 2003), to look for redundancy, and to

evaluate the likelihood of particular metabolic routes (Schuster et al., 2000).

Most of the techniques mentioned above were developed to study microbial systems under physiological steady state by growing them in chemostat bioreactors. While some of these techniques can be applied to plant cell cultures as well, intact plants and their tissues generally do not maintain metabolic steady state long enough. Kinetic modelling is the most generally accepted approach to quantitatively evaluate the dynamics of biological systems. Each of the involved metabolic processes and transport steps can be described using differential equations linking the concentrations of the involved compounds to the actual turnover or transport rates via reaction rate constants. Depending on the underlying reaction mechanism (e.g., Michaelis–Menten, Ping–Pong, or Ternary-complex mechanisms) different rate equations can be applied describing the relation between turnover rate and concentrations of the involved compounds (Bisswanger, 2008). These kinetic models are strongly focussed towards deterministic processes while gene expression (transcription and translation) has largely shown to be of a stochastic nature (McAdams and Arkin, 1997). Kinetic models can also be used to describe the flux of isotope labels through a metabolic network allowing the detailed study of complex networks containing branching and oscillating pathways (Roessner-Tunali et al., 2004; Roscher et al., 2000). In this way, the molecular flux and turnover can be tracked even if total levels of specific metabolites remain unchanged. Network flux models that are based on experimental data (Baxter et al., 2007) or theoretical (Steuer et al., 2003) information can be interpreted against known metabolic pathway structures.

4.4. Model reduction

Processes in biological systems occur at a range of scales, both in terms of size, location, time and complexity. When focussing on whole plant physiology, or as in the case of postharvest the physiology of harvested plant parts (fruits and vegetables), the system behaviour of interest is the end result of a long chain of events starting at the level of molecular interactions determining cellular behaviour which, taking into account differences between the various cell types, affects tissue functioning and finally whole plant physiology. As postharvest data are inevitably characterised by large biological variation (Hertog et al., 2007), stochastic and spatio-temporal variation should ideally be incorporated at all of these functional levels accounting for heterogeneity in chemical and physical properties. Although the reaction-based view of systems biology is appealing in its emphasis on mechanism and dynamics, it is ill-suited to describe whole plant physiology as many reactions are still unknown leading to un-identifiable models (Sorger, 2005). However, by proper lumping, model complexity can be reduced, still conserving the original model performance as well as possible, providing insights into the key species determining the kinetics of the underlying kinetics (Dokoumetzidis and Aarons, 2009).

Additionally, by using multi-scale models it becomes possible to combine detailed reaction-based and large-scale systems-theoretical views of a complex process. Multi-scale models allow integration of information from different spatio-temporal scales while capturing both continuous deterministic processes and discrete stochastic processes (Sorger, 2005). An early multi-scale approach in systems biology by Chen et al. (2004b) modelled the cell growth and division cycle of budding yeast based on a kinetic mechanism that is almost fully specified at the genetic level combining continuous and discrete events successfully describing phenotypes of 120 existing mutants and predicting phenotypes of new mutant combinations.

4.5. Modelling tools

Many initiatives around the world have developed mathematical and statistical approaches in combination with the dedicated software tools to bring the expertise of modelling within reach of the experimental researcher. Over the last decade efforts have been put towards bringing all these initiatives together through the development of a common exchange language which resulted in the Systems Biology Markup Language (SBML), an open source computer-readable format for representing models of biological processes. SBML can be used to formulate models on general metabolism, cell signalling, and other processes, and has been widely adopted by over 180, mostly free, software packages in the area of systems biology (http://sbml.org/SBML_Software_Guide/SBML_Software_Matrix). Thanks to SBML, models can easily be transferred between the various software tools, shared between authors and reused for various purposes. For instance, pathways from the KEGG database can directly be imported into SBML minimising errors when building complex network models. This has opened the door for the development of detailed virtual cell (Moraru et al., 2008), plant (Katari et al., 2010) and even human models (Kohl and Noble, 2009). By combining these largely biochemical and regulatory models with realistic geometry models (Verboven et al., 2008) to account for spatial differences and biophysical models to describe heat and mass transfer (Ho et al., 2009, 2008) or other biophysical processes such as mechanical deformation (Ghysels et al., 2009; Loodts et al., 2006), even more complete models can be obtained of biological subsystems like fruit organs which is the ultimate goal for applications in postharvest systems biology.

5. Potential for postharvest

Why should a postharvest systems approach be of value? If we consider the principal responses and processes, we are increasingly aware that single pathways and responses do not provide an adequate explanation of fruit and vegetable postharvest behaviour. Ripening itself is an integrated process including among other things, respiratory changes, cell wall changes, aroma volatile production, skin colour changes, and control in varying ways by ethylene and other growth regulators. When we add the complications of variable postharvest temperatures and atmosphere conditions, pathogen attack, and specific treatments such as those used for disinfestation, then we can recognise that we are dealing with a matrix or network of responses and processes coordinated in different ways in different crop species. The potential for postharvest can be summarised in four areas.

5.1. Regulation of processes and responses

There has been a huge effort over the years to identify and characterise single events, metabolites or genes which might be part of, or control, the physiological response of a fruit or vegetable. In the end many of these are inadequate because: (1) they often concentrate on only one member of a gene or enzyme family, or metabolic pathway; (2) evidence of a role for genes, proteins, or metabolites is usually only correlative; (3) they rarely take into account the connectivity between genes, pathways or metabolites and we know little on how one gene or enzyme influences another in postharvest responses. With the availability of new technologies, there is a change from single gene or single metabolite approaches to analysis of multiple metabolites, pathways, and gene and protein families. At each step from gene through to metabolite and physiological response we are now confronted with multiple data sets and concepts, not single events or entities. Transcription factors are per-

haps a notable exception. One example of the latter is the control of red pigmentation by members of the MYB transcription factor family, where for instance, red colour in many fruit is regulated by genes such as a *MYB10* in apple fruit, and colour variants can be traced to mutations in these genes (Allan et al., 2008). However, identification of controlling elements for common postharvest responses such as chilling injury or response to low oxygen is a long way behind. This is because these are not single gene or single pathway responses, and a systems approach through knowledge of expression of genes associated with, for instance, antioxidants and phenolic metabolism, combined with metabolomics and physiological measurements (the *physiome*) is likely to be the only way in which we can gain a useful understanding of these processes. A start in this regard has been made with recent studies, particularly with citrus, on global gene expression or proteomics in low temperature (e.g., Yun et al., 2010) or anaerobic (e.g., Shi et al., 2008) conditions, and in modelling CA and browning in pears and apples (Franck et al., 2007; Pedreschi et al., 2008). It is in these complex responses that a systems approach will provide the greatest benefit.

5.2. Quality prediction

Modelling, and particularly systems modelling, does not only increase our understanding of processes and their regulation, but also is inevitably directed to prediction of behaviour. This should allow design of more effective postharvest systems and processes, and perhaps the low level of uptake to date reflects the limitations of using confined datasets for model development. This is now changing, as can be seen for instance on modelling biological variability (see Hertog et al., 2007, and references therein), using enzyme theory to model quality (e.g., Bobelyn et al., 2006), and in modelling specific quality properties such as firmness of fresh-cut tomato (Schouten et al., 2010), or glucosinolates in broccoli (Schouten et al., 2009). Extending these studies to incorporate a wider range of activity and data is now possible, and more accurate predictions of postharvest responses should eventually. Can we expect soon to be able to collect data at harvest, add postharvest storage conditions, and accurately predict consumer flavour perception 6 months later? Current research on human gene responses and variability might also allow us to say which consumers will perceive what aromas (Jaeger et al., 2010).

5.3. Plant improvement

Although fruit quality properties important to postharvest responses, such as texture, taste, and susceptibility to storage disorders, have been among the target traits used for some time in fruit and vegetable breeding, genetic markers for such traits have rarely been successfully used. Genomic information, particularly whole genome sequencing will change our approach to genetic markers, with a de-emphasis on QTLs and random sequences. Greater efforts will be made to identify specific genes for selection and biomarkers which may involve proteins and metabolites. Genard et al. (2007) have pointed out the advantages a model might provide where QTLs for model parameters may be more beneficial than those just for traits, presumably because the model has incorporated a wider set of variables and may take into account environmental effects on trait development. A systems-based model will provide even more rigorous targets because of the broader and deeper sets of information incorporated. Metabolic pathway models can also be used to identify bottlenecks in the biosynthesis of important plant metabolites such as aroma volatiles. This information could guide future breeding efforts.

5.4. Virtual fruit

One goal that is rapidly becoming a reality is the concept of the virtual fruit. In essence this is a systems model which incorporates all critical properties of an organ such as a fruit to demonstrate a co-ordinated response to external environment and the internal, genetically based processes of growth and development. A peach fruit model has been developed based on dry and fresh mass and sugar accumulation and largely concentrating on fruit development (Lescouret and Genard, 2005), and is predominantly physiological and mechanistic. There have been few attempts to incorporate genetic control into fruit models, particularly with regard to quality, and not in terms of postharvest responses. Where this has been attempted, it has been largely at the level of QTLs rather than at the specific gene level (see Genard et al., 2007, and references therein). The information we now have available means that a system approach incorporating genomic, proteomic and metabolomic information with physiological and biophysical data and models, is becoming possible. Postharvest science is notable for the very large archive of datasets available on fruit physiology and postharvest responses in relation to quality in model fruit such as tomato, apple and peach. One of the major challenge in postharvest physiology will thus become the interpretation of 'omics' studies in combination with phenotypic data, and the transformation of large databases into useful information and information into new biological knowledge. The human *physiome* project (<http://www.physiome.org.nz/>; "a worldwide public domain effort to provide a computational framework for understanding human and other eukaryotic physiology. It aims to develop integrative models at all levels of biological organisation, from genes to the whole organism via gene regulatory networks, protein pathways, integrative cell function, and tissue and whole organ structure/function relation") perhaps gives the postharvest world a lead in what might be achieved in a new era.

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